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(54) **Biologically active WS 6049 substances, a process for the production thereof and their pharmaceutical compositions.**

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D scription

This invention relates to new compounds having biological activities, her inafter r ferred to as WS 6049 substances. More particularly, this inventi n relates to a new biologically activ WS 6049-A substance and WS 6049-B substance, which have antimicrobial activities against various path genic microorganism and antitumor activities, to a process for the preparation thereof and to pharmaceutical compositions containing the same.

Accordingly, it is one object of this invention to provide new WS 6049 substances which are active against various pathogenic microorganisms and tumors, and useful for the therapeutical treatment of infectious diseases and of various cancers in human beings and animals.

Another object of this invention is to provide a process for the production of WS 6049 substances by fermentation.

A further object of this invention is to provide a pharmaceutical compositions containing, as an active ingredient, WS 6049 substances.

In the preceding and following descriptions, it is to be noted that the wording "WS 6049 substances" is intended to include WS 6049-A substance and WS 6049-B substance.

WS 6049 substances of this invention can be produced by fermentation of WS 6049 substance-producing strain *Actinomadura pulveraceus* sp. nov. No. 6049 (ATCC 39100) in a nutrient medium.

Particulars of the microorganism used for producing WS 6409 substances and production thereof will be explained in the following.

The microorganism

The microorganism which can be used for the production of WS 6049 substances is the strain *Actinomadura pulveraceus* sp. nov. No. 6049 which has been newly isolated from a soil sample collected at Wakayama City in Wakayama Prefecture, Japan.

A culture of the newly isolated living organism of *Actinomadura pulveraceus* sp. nov. No. 6049 has been deposited with and added to a stock culture collection of the American Type Culture Collection under the Budapest Treaty and its deposit number is ATCC 39100 (deposit date: April 12, 1982).

Actinomadura pulveraceus sp. nov. No. 6049 has the following morphological, cultural and physiological characteristics.

(1) Morphological characteristics:

The methods described by Shirling and Gottlieb were employed for observation of the morphological characteristics (Shirling, E. B. and D. Gottlieb: Methods for characterization of *Streptomyces* species, Int. J. Syst. Bacteriol. 16: 313—340, 1966).

Morphological observations were made with light and electron microscopy on cultures grown at 30°C for 21 days on yeast extract-malt extract agar, inorganic salts-starch agar and oatmeal agar. The mature spores occurred in chains of 5 to 20 spores forming mainly hook and sometimes loose spirals. The spores were oval and 0.8—1.0×1.2—1.4 μ in size with warty surfaces.

(2) Cultural characteristics:

Cultural characteristics were observed on ten kinds of media described by Shirling and Gottlieb (mentioned hereinabove) and Waksman (Waksman, S. A.: The Actinomycetes, Vol. 2. Classification, identification and description of genera and species. The Williams and Wilkins Co., Baltimore, 1961). The incubation was made at 30°C for 21 days. The color names used in this study were based on Color Standard (Nihon Shikisai Co., Ltd.). As shown in Table 1, colonies belonged to the blue color series when the strain was grown on yeast extract-malt extract agar, oatmeal agar and inorganic salts-starch agar. Soluble pigment was not produced.

The cell-wall composition analysis was performed by the methods of Beker et al. (Becker, B., M. P. Lechevalier, R. E. Gordon and H. A. Lechevalier: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates, Appl. Microbiol. 12, 421—423, 1964) and Yamaguchi (Yamaguchi, T.: Comparison of the cell-wall composition of morphologically distinct actinomycetes, J. Bacteriol. 89, 444—453, 1965). The cell wall of this strain No. 6049 contained *meso*-diaminopimelic acid. A whole cell hydrolysate showed the presence of glucose, mannose and madurose (3 - O - methyl - D - galactose).

(3) Biological and physiological properties:

Temperature range for growth and optimum temperature were determined on yeast extract-malt extract agar using temperature gradient incubator (Toyo Kagaku Sangyo Co., Ltd.). The pH range for growth and the optimum pH were determined by liquid cultivation in yeast extract-malt extract broth with shaking at 30°C for 7 days. Gelatin liquefacti n was examined at 30°C for 14 days on gelatin medium. Starch hydrolysis was bserved by the starch-iodin reacti n after incubation on inorganic salts-starch agar plat at 30°C for 14 days. Milk pept nizati n was bserved in skim-milk medium at 30°C for 14 days. M lanoid pigment producti n was observed on tyrosin agar, peptone-yeast extract-iron agar and trypt ne-y ast extract broth. As shown in Tabl 2, temp rature range for growth was from 20°C to 41°C with optimum

from 30°C to 35°C. Starch hydr lysis, milk p ptonization, H_2S producti n, urease activity, nitrate reduction and melamine production were n gative, wher as gelatin liqu faction and milk coagulation were positive.

Utilization of carb n sources was examined acc rding to the m th d of Pridham and Gottli b (Pridham, T. G. and D. Gottli b: The utilizati n of carbon compounds by some Actinomycetales as an aid for species determination, J. Bacteriol. 56: 107—114, 1965). The results were determined after 14 days incubation at 30°C. As shown in Table 3, almost all carbon sources were doubtfully or not utilized. Only D-xylose, D-glucose and D-trehalose were utilized. The aforementioned cell wall composition and whole cell sugar components indicate the strain No. 6049 is a species of the genus *Actinomadura*..

A comparison of this organism was made with the published descriptions of *Actinomadura* species (Nonomura, H. and Y. Ohara: Distribution of actinomycetes in soil, XI). Some new species of the genus *Actinomadura* Lechevalier et al., J. Ferment. Technol. 49: 904—912, 1971; Goodfellow, M., G. Alderson and J. Lacey: Numerical taxonomy of *Actinomadura* and related actinomycetes, J. Gen. Microbiol. 112: 95—111, 1979, Tomita, K., Y. Hoshino, T, Sasahira and H. Kawaguchi: BBM-928, a new antitumor antibiotic complex 2. Taxonomic studies on the producing organism, J. Antibiotics 33: 1098—1102, 1980).

Strain No. 6049 is considered to resemble *Actinomadura verrucosospora*. It was found however, that strain No. 6049 could be differentiated from this species in the following points. As shown in Table 4, utilization of D-fructose, L-arabinose, mannitol, sucrose and glycerin is different. Differences are also observed in nitrate reduction and milk peptonization. Aerial mass color of strain No. 6049 belonged to the white color series on inorganic salts-starch agar, whereas that of *A. verrucosospora* belonged to the gray color series. Direct comparison of cultural characteristics between strain No. 6049 and *A. verrucosospora* was shown in Table 5.

As a result of above comparisons, strain No. 6049 is considered a new species of the genus *Actinomadura*. The name *Actinomadura pulveraceus* sp. nov. is proposed for strain No. 6049 referring to the powdery aerial mycelium on yeast extract-malt extract agar, oatmeal agar and inorganic salts-starch agar.

TABLE 1
Cultural characteristics of strain No. 6049

Medium	Aerial mycelium	Reverse side color	Soluble pigment
Oatmeal agar	greenish white	colorless	none
Yeast extract-malt extract agar	pale blue	pale yellow	none
Inorganic salts-starch agar	white	light red	none
Glucose asparagine agar	none	pink	none
Glycerin-asparagine agar	trace	pale pink	none
Sucrose-nitrate agar	none	pale pink	none
Nutrient agar	none	light red	none
Potato-dextrose agar	none	pale pink	none
Tyrosine agar	grayish white	pale pink	none
Peptone-yeast extract-iron agar	none	pale pink	none

TABLE 2
Physi logical properties of strain No. 6049

Temperature rang for growth	20°C—41°C
Optimum temperature	30°C—35°C
pH range for growth	6—10
Optimum pH	7—8
Nitrate reduction	negative
Starch hydrolysis	positive
Milk coagulation	weakly positive
Milk peptonization	negative
Gelatin liquefaction	positive
Melanine production	negative
H_2S production	negative
Urease	negative

TABLE 3
Carbon sources utilization of strain No. 6049

C-sources	
none	−
Glycerin	±
D-Xylose	+
Sodium citrate	−
Lactose	−
D-Fructose	−
Rhamnose	+
Maltose	±
Sodium succinate	−
Inulin	−
Inositol	±
Raffinose	−
D-Galactose	±
L-Arabinose	−
D-Glucose	+
Mannitol	−
D-Mannose	−
Sucrose	+
Cellulose	−
D-Trehalose	−
Salicin	−
Chitin	−
Sodium acetate	−

+: utilization
±: doubtful utilization
−: not utilization

TABLE 4
Differ nt p ints of carbon utilization and physiological properties betwe n strain No. 6049 and *Actinomadura verrucosospora*

	No. 6049	*A. verrucosospora*
D-Fructose	−	+
L-Arabinose	−	+
Mannitol	−	+
Sucrose	++	±
Glycerin	±	++
Nitrate reduction	negative	positive
Milk peptonization	negative	positive

++: good utilization
+: utilization
±: doubtful utilization
−: not utilization

TABLE 5
Direct comparison between strain No. 6049 and *Actinomadura verrucosospora*

Medium	Strain No. 6049			
	Growth	Aerial mycelium	Reverse side color	Soluble pigment
1	abundant	none	pale yellow	none
2	poor	none	colorless	none
3	abundant	blue	pink	none
4	abundant	white	pink	none
5	moderate	grayish white	colorless	none
6	abundant	none	colorless	none
7	poor	grayish white	colorless	none
8	moderate	pale blue	pale pink	none
9	abundant	pinkish grey	pink	none

TABLE 5 (contd.)

Medium	*Actinomadura verrucosospora* Growth	Aerial mycelium	Reverse side color	Solubl pigment
1	moderate	none	pink	none
2	moderate	none	pink	none
3	poor	none	pink	none
4	poor	none	pale pink	none
5	moderate	grayish white	pink	none
6	abundant	none	pink	none
7	poor	grayish blue	pink	none
8	abundant	pale bluish pink	pink	none
9	abundant	pale bluish pink	pink	none

1: Peptone-yeast extract-iron agar,
2: Nutrient agar,
3: Potato-dextrose agar,
4: Sucrose-nitrate agar,
5: Tyrosine agar,
6: Yeast extract-malt extract agar,
7: Oatmeal agar,
8: Inorganic salts-starch agar,
9: Glucose-asparagine agar.

Production of WS 6049 substances

The WS 6049 substances of this invention are produced when the WS 6049 substance-producing strain *Actinomadura pulveraceus* sp. no. No. 6049 is grown in a nutrient medium containing sources of assimilable carbon and nitrogen under aerobic conditions (e.g. shaking culture, submerged culture).

The preferred sources of carbon in the nutrient medium are carbohydrates such as xylose, glucose, sucrose and starch.

The preferred sources of nitrogen are yeast extract, peptone, gluten meal, cotton seed flour, soybean meal, corn steep liquor, dried yeast and wheat germ, as well as inorganic and organic nitrogen compounds such as ammonium salts (e.g. ammonium nitrate, ammonium sulfate, ammonium phosphate), urea and amino acid.

The carbon and nitrogen sources, though advantageously employed in combination, need not be used in their pure form because less pure materials, which contain traces of growth factors and considerable quantities of mineral nutrients, are also suitable for use.

When desired, there may be added to the medium mineral salts such as calcium carbonate, sodium or potassium phosphate, sodium or potassium chloride, magnesium salts and copper salts.

If necessary, especially when the culture medium foams seriously a defoaming agent, such as liquid paraffin, fatty oil, plant oil, mineral oil or silicone may be added.

As in the case of the preferred methods used for the production of other antibiotics in massive amounts, submerged aerobic cultural conditions are preferred for the production of WS 6049 substances in massive amounts.

For the production in small amounts, a shaking or surface culture in a flask or bottle is employed. Furtherm r , when the growth is carried out in larg tanks, it is preferabl to use the vegetative form of the organism for inoculation in the production tanks in ord r to avoid growth lag in the pr c ss of production of the WS 6049 substances. Accordingly, it is desirable first t pr duce a v getative inoculum of the organism by inoculating a relatively small quantity of culture medium with spores or mycelia of the organism and culturing said inoculated m dium, and then t transfer the cultured v getative inoculum

aseptically to larg tanks. The medium, in which th vegetativ inoculum is produced, is substantially the same as or different from the m dium utilized for th production of WS 6049 substances.

Agitation and a ration of the cultur mixture may b accomplish d in a variety of ways. Agitation may be provided by a propeller or similar m chanical agitation equipment, by revolving or shaking the fermentor, by various pumping equipment or by the passage of sterile air through the medium. Aeration may be effected by passing sterile air through the fermentation mixture.

The fermentation is usually conducted at a temperature between about 20°C and 40°C, preferably 25—30°C, for a period of about 50 hours to 100 hours.

When the fermentation is completed, the culture broth is then subjected for recovery of WS 6049 substances to various procedures conventionally used for recovery and purification of antibiotics, for instance, solvent extraction with an appropriate solvent or a mixture of such solvents, chromatography, or recrystallization from an appropriate solvent or a mixture of such solvent.

According to this invention, in general, WS 6049 substances are found mainly in the cultured mycelia. Accordingly, the culture broth is separated by means of filtration or centrifuging to provide the mycelium cake, and then WS 6049 substances are recovered from said resulting mycelium cake by means of extraction using an appropriate organic solvent such as acetone or ethyl acetate, or a mixture of such solvents.

The extract is treated by a conventional manner to provide WS 6049 substances, for example, the extract is concentrated by evaporation or distillation to a smaller amount and the resulting residue containing active materials, i.e. WS 6049 substances are purified by conventional purification procedures, for example, chromatography or recrystallization from an appropriate solvent or a mixture of such solvents.

WS 6049-A substances and WS 6049-B substances can be separated by dissolving the materials containing both product produced by fermentation in an appropriate organic solvent such as chloroform and then by chromatographing the solution, for example, on silica gel in a column with an appropriate organic solvent or a mixture of such solvents such as chloroform or acetone and each of WS 6049-A substance and WS 6049-B substance thus separated can be further purified by a conventional method, for example, high performance liquid chromatography.

Physical and chemical properties of WS 6049 substances

The WS 6049 substances i.e. WS 6049-A substance and WS 6049-B substance as obtained according to the aforementioned process have the following physical and chemical properties.

WS 6049-A

1) Form and color:
   Colorless powder
2) Color reaction:
   Positive: Dragendorff reaction, Ehrlich's reaction and cerium sulfate reaction
   Negative: ninhydrin reaction
3) Solubility:
   Soluble: methanol, acetone, chloroform
   Slightly soluble: diethyl ether
   Insoluble: hexane, water
4) Melting point:
   150°C (dec.)
5) Specific rotation:
   $[\alpha]_D^{25}=-208°$ (c=1.0, $CHCl_3$)
6) Ultraviolet absorption spectrum:
   $\lambda_{max}^{CH_3OH}=252$ nm ($E_{1\,cm}^{1\%}=560$)
   $=280$ nm ($E_{1\,cm}^{1\%}=370$)
   $=320$ nm ($E_{1\,cm}^{1\%}=220$)
   $\lambda_{max}^{CH_3OH+HCl}=252, 280, 320$ nm
   $\lambda_{max}^{CH_3OH+NaOH}=250, 280,$ ca 310 (sh.) nm
7) Infrared absorption spectrum:
   $\nu_{max}^{CHCl_3}$=3450, 3350, 3250, 2960, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1075, 1020, 985, 955, 905, 850 cm^{-1}
8) Elementary analysis:
   C: 52.03%, H: 5.71%, N: 4.15%, S: 9.86%
9) Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel sheet	Chloroform: methanol (10:1 (v/v)	0.59
	Chloroform: acetone (1:1) (v/v)	0.33

10) Molecular weight:
FD Mass: m/s 1333 (M^++Na)
FABQ MS: m/z 1311 (M^++1)
Gel permeation chromatography: 1100 to 1200

11) ^{13}C Nuclear magnetic resonance spectrum ($CDCl_3$):
δ (ppm): 13.9, 14.6, 16.7, 17.6, 19.9, 22.8, 29.1, 34.0, 35.2, 39.6, 42.3, 52.7, 55.8, 56.1, 56.2, 56.2, 60.3, 61.7, 64.7, 66.7, 68.4, 69.0, 69.3, 69.8, 70.5, 72.0, 75.9, 76.1, 77.2, 77.3, 83.2, 86.3, 88.5, 90.7, 97.3, 98.6, 98.9, 99.6, 99.7, 103.9, 107.8, 112.7, 123.2, 125.0, 130.0, 131.1, 136.8, 144.2, 146.6, 154.0, 154.6, 160.9, 166.6, 192.4, as shown in Figure 1 of the accompanying drawing.

12) ^1H Nuclear magnetic resonance spectrum ($CDCl_3$):
δ (ppm): 8.57 (1H, s), 7.48 (1H, s), 6.6 (1H, d.d), 6.2 (1H, d, J=1.6Hz), 6.18 (1H, br.s.), 5.93 (1H, d, J=9.6Hz), 5.83 (1H, d.d J=9.6, 1.6Hz), 5.7 (1H, d, J=2Hz), 5.5 (1H, m), 5.48 (1H, d, J=2.3Hz), 5.43 (1H, br.s), 4.97 (1H, d), 4.7 (2H, m), 4.56 (1H, d, J=2.3Hz), 4.23 (1H, s), 4.2 to 3.6 (10 to 14H), 3.97 (3H, s), 3.88 (3H, s), 3.79 (3H, s), 3.5 (2H, m), 3.43 (3H, s), 2.77 (1H, s), 2.7 (2H, m), 2.52 (3H, s), 2.5 (1H, m), 2.4 to 2.25 (3H, m), 2.17 (1H, s), 2.12 (3H, s), 2.07 (1H, m), 1.77 (2H, s), 1.5 (2H, m), 1.41 (3H, d, J=6Hz), 1.35 (3H, d, J=6Hz), 1.32 (3H, d, J=6Hz), 1.2 (4H, m), as shown in Figure 2 of the accompanying drawing.

WS 6049-B

1) Form and color:
Colorless powder

2) Color reaction:
Positive: Dragendorff reaction, Ehrlich's reaction and cerium sulfate reaction
Negative: ninhydrin reaction

3) Solubility:
Soluble: methanol, acetone, chloroform
Slightly soluble: diethyl ether
Insoluble: hexane

4) Melting point:
145°C (dec.).

5) Specific rotation:
$[\alpha]_D^{25}=-201°$ (c=1.0, $CHCl_3$)

6) Ultraviolet absorption spectrum:
$\lambda_{max}^{CH_3OH}$=253 nm ($E_{1cm}^{1\%}$=620)
=280 nm ($E_{1cm}^{1\%}$=450)
=320 nm ($E_{1cm}^{1\%}$=250)
$\lambda_{max}^{CH_3OH+HCl}$=253, 280, 320 nm
$\lambda_{max}^{CH_3OH+NaOH}$=250, 282, 310 (sh.) nm

7) Infrared absorption spectrum:
$\nu_{max}^{CHCl_3}$=3450, 3350, 3250, 2990, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1070, 1020, 985, 955, 905, 880, 850 cm^{-1}

8) Elementary analysis:
C: 61.58%, H: 5.75%, N: 4.27%, S: 9.80%

9) Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel sheet	chloroform: methanol (10:1 (v/v)	0.53
	chlorof rm: acet ne (1:1) (v/v)	0.18

10) Molecular w ight:
Gel permeation chromatography: 1100 to 1200

11) ^{13}C Nuclear magn tic resonance spectrum ($CDCl_3$):
δ (ppm): 13.8, 16.7, 17.6, 19.8, 22.7, 29.1, 33.9, 34.1, 35.2, 39.5, 52.8, 55.8, 56.1, 56.3, 61.0, 61.5, 64.6, 66.7, 68.4, 69.0, 69.3, 69.8, 70.4, 71.9, 75.9, 76.2, 76.7, 77.2, 77.3, 77.6, 83.2, 86.6, 88.3, 90.7, 97.3, 98.6, 99.0, 99.6, 99.7, 103.9, 107.8, 112.7, 123.2, 124.9, 129.8, 131.0, 136.8, 144.2, 146.3, 154.0, 154.6, 160.9, 166.6, 192.4, as shown in Figure 3 of the accompanying drawing.

12) ^{1}H Nuclear magnetic resonance spectrum ($CDCl_3$):
δ (ppm): 11.75 (1H, s), 8.57 (1H, s), 7.48 (1H, s), 6.6 (1H, d.d.), 6.24 (1H, d, J=1.3Hz), 6.18 (1H, br.s), 5.93 (1H, d, J=9.2Hz), 5.83 (1H, d.d, J=9Hz and 1.3Hz), 5.70 (1H, br.d), 5.5 (1H, m), 5.47 (1H, d, J=2.3Hz), 5.42 (1H, br.s), 4.98 (1H, d, J=9Hz), 4.70 to 4.6 (2H, m), 4.56 (1H, d, J=2.3Hz), 4.24 (1H, s), 4.15 to 3.4 (12 to 18H), 3.97 (3H, s), 3.88 (3H, s), 3.79 (3H, s), 3.42 (3H, s), 2.52 (3H, s), 2.6 to 2.4 (2H, m), 2.4 to 2.2 (7 to 8H), 2.12 (3H, s), 2.2 to 2.0 (2H, m), 1.5 (2H, m), 1.4 (3H, d, J=6Hz), 1.35 (3H, d, J=6Hz), 1.33 (3H, d, J=6Hz), as shown in Figure 4 of the accompanying drawing.

Biological properties of WS 6049 substances

Biological properties of WS 6049 substances are explained in the following.

1) Antitumor activities of WS 6049 substances:

The antitumor activities of WS 6049 substances were determined in experimental tumor system in mice.

Lymphocytic leukemia P-388 was implanted intraperitoneally into BDF_1 mice at an inoculum size of 1×10^6 cells per mouse. Twenty-four hours after the implantation of tumor cells, graded doses of the antibiotics were administered to mice intraperitoneally. Treatments were given on day 1, 2, 3 and 4.

The WS 6049 substances were suspended in physiological saline solution (0.9% saline). Control animals received intraperitoneal doses of physiological saline solution. The injection volume was 0.2 ml in all experiments.

The therapeutic responses measured was mean survival time, and results were expressed as T/C% (survival time of treated group/survival time of control group×100). Toxicity was measured as weight loss between Days 0 and 4 after tumor inoculation.

The result is presented in Table 6 and Table 7. The WS 6049-A and WS 6049-B were quite active against the leukemia P-388. Doses between 0.025—12.0 μg/kg on the schedule resulted in significant increase in life span in mice.

TABLE 6
P-388 m use leukemia
BDF$_1$ mouse (♀, 7 week); tumor site i.p. (1×10^6 cells/mouse, day 0); drug route i.p. (day 1, 2, 3 and 4); fiv mice per group

Drug	Dose (μg/kg/day)	Weight change (g) day 0—day 4	Mean survival time (days)	T/C%
WS 6049-A	25 μg/kg/day	−3.1	7.6	toxic
	12	−2.0	16.4	173 (1/5)*
	6	+0.1	21.5	226
	3	+0.2	21.5	226 (1/5)*
	1.5	+0.2	22.5	237
	0.8	+0.3	22.0	232
	0.4	+0.2	21.6	227
	0.2	+0.2	20.0	211
	0.1	+0.2	18.6	196
	0.05	+0.1	16.6	175
	0.025	+0.6	12.3	129
Control	—	+1.0	9.5	100

$*: \dfrac{\text{Numbers of survivor at day 30}}{\text{Total mice}}$

TABLE 7
P-388 mouse leukemia
BDF$_1$ mouse (♀, 7 week); tumor site i.p. (1×10^6); drug route i.p. (day 1, 2, 3 & 4); five mice per group

Drug	Dose	Weight change (g) day 0—day 4	Mean survival time (days	T/C%
WS 6049-B	10 μg/kg/day	−1.7	8.9	72
	3.3	−0.4	26.4	215
	1.1	−1.2	20.7	168
	0.37	+0.3	23.3	189
	0.12	0	21.9	178
	0.04	+0.5	24.8	202
	0.013	+0.6	17.7	144
Control	vehicle 0.2 ml/ mouse/day	+0.6	12.3	100

(2) Antimicrobial activiti s of WS/6049-A and WS/6049-B

Antimicrobial activites of WS/6049-A and WS/6049-B wer determined by a serial br th dilution method in bouillon medium for bacteria and in Sabouraud medium f r fungi and yeast. Minimum inhibitory concentrations (MIC) were expressed in terms of µg/ml after overnight incubation at 37°C for bacteria and 48—72 hours incubation at 28°C for fungi and yeasts. The antimicrobial spectra of WS 6049-A and WS 6049-B are shown in Table 8.

From the results, the antibiotics, WS 6049 substances have a broad antimicrobial activity and may be among the most potent antibiotics ever discovered.

TABLE 8
Antimicrobial spectra of WS 6049-A and WS 6049-B

Microorganism	MIC (µg/ml)	
	WS 6049-A	WS 6049-B
Staphylococcus aureus 209 P	0.0001—0.0002	0.0001—0.0002
Bacillus subtilis	0.001	0.001
Escherichia coli	0.6	0.6
Pseudomonas aeruginosa	0.6	0.6
Proteus vulgaris	1.2	1.2
Candida albicans	5.0	5.0
Aspergillus oryzae	0.6	0.6
Penicillium chrysogenum	0.3	0.3
Aureobasidium sp.	0.15	0.15

3) Acute toxicity of WS 6049-A and WS 6049-B

Acute toxicity of WS 6049-A and B in ddY mice by intraperitoneal injection are both 0.05 mg/kg.

The pharmaceutical composition of this invention can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid or liquid form, which contains WS 6049 substances, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for external, enteral or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form, and in addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used. The active object compound is included in the pharmaceutical composition in an amount sufficient to produce the desired antimicrobial effect upon the process or condition of diseases.

For applying this composition to humans, it is preferably to apply it by intravenous, intramuscular or oral administration. While the dosage or therapeutically effective amount of the object compound of this invention varies from and also depends upon the age and condition of each individual patient to be treated, a daily dose of about 0.01—50 µg of the active ingredient/kg of a human being or an animal is generally given for treating diseases, and an average single dose of about 0.1 µg, 1 µg, 10 µg, 50 µg, 100 µg and 200 µg is generally administered.

The following example is given for the purpose of illustrating the present invention.

Example

An aqueous medium (160 ml) containing 2% starch, 0.5% glucose, 1% cotton seed flour, 1% dried yeast, 0.5% corn steep liquor and 0.2% $CaCO_3$ (pH 7.0) was poured into each of three 500 ml Erlenmeyer flasks and st rilized at 120°C f r 30 minutes. A loopful of slant culture f *Actinomadura pulveraceus* sp. nov. N . 6049 (ATCC 39100) was in culated to each of the medium and cultured at 30°C n a rotary shaker with 3-inch throw at 220 rpm for 4 days. The r sultant culture was inoculated to an aque us medium (20 liters) containing 4% sucros , 0.5% dried y ast, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl, 0.2% $(NH_4)_2SO_4$, 0.2% $CaCO_3$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0001% $MnCl_2 \cdot 4H_2O$, 0.0001% $ZnSO_4 \cdot 7H_2O$ and 0.00005% of NaI in

a 30 liter jar-f rmentor, which has been sterilized at 120°C for 30 minutes, and cultured at 30°C for 4 days under aeration of 20 liters/minute and agitation of 300 r.p.m.

The cultured broth thus obtained was filt r d with an aid of diatomaceous earth (1 kg). To the mycelia btained were added 10 liters of ethyl acetate and stirred for 10 minutes. This extraction procedure was carried out twice and the extracts were combined. The extracts w re washed with 10 liters of 1% sodium bicarbonate and 10 liters of 10% sodium chloride. Then the extracts were concentrated *in vacuo* to a volume of one liter. After dehydration with anhydrous sodium sulfate, the ethyl acetate solution was further concentrated *in vacuo* and the oily materials obtained were applied to a column chromatography using silica gel (70 ml). The column was washed with 200 ml of chloroform and eluted with a mixture of chloroform-methanol (40:1). Fractions containing active materials (500 ml) were concentrated *in vacuo* to give a crude powder (140 mg).

The powder was dissolved into 2 ml of chloroform and subjected to a column of silica gel (20 ml). The column was washed with a mixture of chloroform-acetone (8:1), and WS 6049-A was eluted with a mixture of chloroform-acetone (4:1). WS 6049-B was eluted with a mixture of chloroform-acetone (2:1).

Each of fractions containing WS 6049-A and WS 6049-B was concentrated *in vacuo* to give a crude powder of WS 6049-A (17 mg) and WS 6049-B (15 mg), respectively. They were separately subjected to high performance liquid chromatography (HPLC). HPLC was carried out using a Waters Model 6000 A pump with a Waters Model U6K injector. Chromatography was monitored by a UV detector, Waters Model 440 at 254 nm. A steel column (7.9 mm inside diameter, 300 mm length) packed with a μ Porasil (Merck, Darmstadt) was used at a flow rate of 3 ml/minutes. Mobile phase used was a mixture of hexane, chloroform and methanol (25:10:2). HPLC under the above-mentioned conditions gave fraction A (retention time: 18 min) and fraction B (retention time: 26 min). Twelve mg of colorless powder of WS 6049-A from fraction A and 8 mg of colorless powder of WS 6049-B from fraction B were obtained.

Claims

1. WS 6049 substances selected from WS 6049-A substance and WS 6049-B substance, wherein

i) the WS 6049-A has the following properties:

1) Form and color:
   Colorless powder
2) Color reaction:
   Positive: Dragendorff reaction, Ehrlich's reaction and cerium sulfate reaction
   Negative: ninhydrin reaction
3) Solubility:
   Soluble: methanol, acetone, chloroform
   Slightly soluble: diethyl ether
   Insoluble: hexane, water
4) Melting point:
   150°C (dec.)
5) Specific rotation:
   $[\alpha]_D^{25}=-208°$ ($C=1.0$, $CHCl_3$)
6) Ultraviolet absorption spectrum:
   $\lambda_{max}^{CH_3OH}=252$ nm ($E_{1cm}^{1\%}=560$)
   $=280$ nm ($E_{1cm}^{1\%}=370$)
   $=320$ nm ($E_{1cm}^{1\%}=220$)
   $\lambda_{max}^{CH_3OH+HCl}=252, 280, 320$ nm
   $\lambda_{max}^{CH_3OH+NaOH}=250, 280$, ca 310 (sh.) nm
7) Infrared absorption spectrum:
   $\nu_{max}^{CHCl_3}=3450, 3350, 3250, 2960, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1075, 1020, 985, 955, 905, 850\ cm^{-1}$
8) Elementary analysis:
   C: 52.03%, H: 5.71%, N: 4.15:, S: 9.86%
9) Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel sheet	chloroform: methanol (10:1) (v/v)	0.59
	chloroform: acetone (1:1) (v/v)	0.33

10) Molecular weight:
FD Mass: m/z 1333 (M^++Na)
FABQ MS: m/z 1311 (M^++1)
Gel permeation chromatography: 1100—1200

11) ^{13}C Nuclear magnetic resonance spectrum ($CDCl_3$):
δ (ppm): 13.9, 14.6, 16.7, 17.6, 19.9, 22.8, 29.1, 34.0, 35.2, 39.6, 42.3, 52.7, 55.8, 56.1, 56.2, 56.2, 60.3, 61.7, 64.7, 66.7, 68.4, 69.0, 69.3, 69.8, 70.5, 72.0, 75.9, 76.1, 77.2, 77.3, 83.2, 86.3, 88.5, 90.7, 97.3, 98.6, 98.9, 99.6, 99.7, 103.9, 107.8, 112.7, 123.2, 125.0, 130.0, 131.1, 136.8, 144.2, 146.6, 154.0, 154.6, 160.9, 166.6, 192.4, as shown in Figure 1 of the accompanying drawing.

12) ^1H Nuclear magnetic resonance spectrum ($CDCl_3$):
δ (ppm): 8.57 (1H, s), 7.48 (1H, s), 6.6 (1H, d.d), 6.2 (1H, d, J=1.6Hz), 6.18 (1H, br.s.), 5.93 (1H, d, J=9.6Hz), 5.83 (1H, d.d J=9.6, 1.6Hz), 5.7 (1H, d, J=2Hz), 5.5 (1H, m), 5.48 (1H, d, J=2.3Hz), 5.43 (1H, br.s), 4.97 (1H, d), 4.7 (2H, m), 4.56 (1H, d, J=2.3Hz), 4.23 (1H, s), 4.2 to 3.6 (10 to 14H), 3.97 (3H, s), 3.88 (3H, s), 3.79 (3H, s), 3.5 (2H, m), 3.43 (3H, s), 2.77 (1H, s), 2.7 (2H, m), 2.52 (3H, s), 2.5 (1H, m), 2.4 to 2.25 (3H, m), 2.17 (1H, s), 2.12 (3H, s), 2.07 (1H, m), 1.77 (2H, s), 1.5 (2H, m), 1.41 (3H, d, J=6Hz), 1.35 (3H, d, J=6Hz), 1.32 (3H, d, J=6Hz), 1.2 (4H, m), as shown in Figure 2 of the accompanying drawing, and

ii) the WS 6049-B substance has the following properties:

1) Form and color:
Colorless powder

2) Color reaction:
Positive: Dragendorff reaction, Ehrlich's reaction and cerium sulfate reaction
Negative: ninhydrin reaction

3) Solubility:
Soluble: methanol, acetone, chloroform
Slightly soluble: diethyl ether
Insoluble: hexane

4) Melting point:
145°C (dec.).

5) Specific rotation:
$[\alpha]_D^{25}=-201°$ (C=1.0, $CHCl_3$)

6) Ultraviolet absorption spectrum:
$\lambda_{max}^{CH_3OH}=253$ nm ($E_{1cm}^{1\%}=620$)
$=280$ nm ($E_{1cm}^{1\%}=450$)
$=320$ nm ($E_{1cm}^{1\%}=250$)
$\lambda_{max}^{CH_3OH+HCl}=253$, 280, 320 nm
$\lambda_{max}^{CH_3OH+NaOH}=250$, 282, 310 (sh.) nm

7) Infrared absorption spectrum:
$\nu_{max}^{CHCl_3}=3450$, 3350, 3250, 2990, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1070, 1020, 985, 955, 905, 880, 850 cm^{-1}

8) Elementary analysis:
C: 51.58%, H: 5.75%, N: 4.27%, S: 9.80%

9) Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel sheet	Chloroform: methanol (10:1) (v/v)	0.53
	Chloroform: acetone (1:1) (v/v)	0.18

10) Molecular weight:
Gel permeation chromatography: 1100 to 1200

11) ^{13}C Nuclear magnetic resonance spectrum ($CDCl_3$):
δ (ppm): 13.8, 16.7, 17.6, 19.8, 22.7, 29.1, 33.9, 34.1, 35.2, 39.5, 52.8, 55.8, 56.1, 56.3, 61.0, 61.5, 64.6, 66.7, 68.4, 69.0, 69.3, 69.8, 70.4, 71.9, 75.9, 76.2, 76.7, 77.2, 77.3, 77.6, 83.2, 86.6, 88.3, 90.7, 97.3, 98.6, 99.0, 99.6, 99.7, 103.9, 107.8, 112.7, 123.2, 124.9, 129.8, 131.0, 136.8, 144.2, 146.3, 154.0, 154.6, 160.9, 166.6, 192.4, as shown in Figure 3 of the accompanying drawing.

12) H Nuclear magnetic resonanc spectrum ($CDCl_3$):
δ (ppm): 11.75 (1H, s), 8.57 (1H, s), 7.48 (1H, s), 6.6 (1H, d.d.), 6.24 (1H, d, J=1.3Hz), 6.18 (1H, br.s),

5.93 (1H, d, J=9.2Hz), 5.83 (1H, d.d, J=9Hz and 1.3Hz), 5.70 (1H, br.d), 5.5 (1H, m), 5.47 (1H, d, J=2.3Hz), 5.42 (1H, br.s), 4.98 (1H, d, J=9Hz), 4.7 to 4.6 (2H, m), 4.56 (1H, d, J=2.3Hz), 4.24 (1H, s), 4.15 to 3.4 (12 to 18H), 3.97 (3H, s), 3.88 (3H, s), 3.79 (3H, s), 3.42 (3H, s), 2.52 (3H, s), 2.6 to 2.4 (2H, m), 2.4 to 2.2 (7 to 8H), 2.12 (3H, s), 2.2 to 2.0 (2H, m), 1.5 (2H, m), 1.4 (3H, d, J=6Hz), 1.35 (3H, d, J=6Hz), 1.33 (3H, d, J=6Hz), as shown in Figure 4 of the accompanying drawing.

2. A process for the production of WS 6049 substances according to claim 1 selected from WS 6049-A substance and WS 6049-B substance which comprises culturing the strain *Actinomadura pulveraceus* sp. nov. No. 6049 (ATCC 39100) in an aqueous nutrient medium under aerobic conditions and recovering WS 6049-A substance and/or WS 6049-B substance.

3. A pharmaceutical composition which comprises, an active ingredient selected from WS 6049-A and WS 6049-B according to claim 1 and a non-toxic, pharmaceutically acceptable carrier.

4. A biologically pure culture of the microorganism *Actinomadura pulveraceus* sp. nov. No. 6049 (ATCC 39100).

5. WS 6049-A substance as defined in claim 1 for treatment of cancer.

6. WS 6049-B substance as defined in claim 1 for treatment of cancer.

7. Use of WS 6049 substances of claim 1 for preparing a drug for treatment of cancer.

Patentansprüche

1. Substanz WS 6049, ausgewählt aus den Substanzen WS 6049-A und WS 6049-B, worin

i) WS 6049-A die nachstehenden Eigenschaften aufweist:

1) Form und Farbe:
farbloses Pulver

2) Farbreaktion:
positiv: Dragendorff-Reaktion, Ehrlich's Reaktion und Cersulfat-Reaktion
negativ: Ninhydrin-Reaktion

3) Löslichkeit:
löslich: Methanol, Aceton, Chloroform,
wenig löslich: Diethylether
unlöslich: Hexan, Wasser

4) Schmelzpunkt:
150°C (Zers.)

5) Spezifische Rotation:
$[\alpha]_D^{25}=-208°$ (C=1,0, $CHCl_3$)

6) UV-Absorptionsspektrum:
$\lambda_{max}^{CH_3OH}=252$ nm ($E_{1cm}^{1\%}=560$)
$=280$ nm ($E_{1cm}^{1\%}=370$)
$=320$ nm ($E_{1cm}^{1\%}=220$)
$\lambda_{max}^{CH_3OH+HCl}=252$, 280, 320 nm
$\lambda_{max}^{CH_3OH+NaOH}=250$, 280, ca. 310 (sh.) nm

7) IR-Absorptionsspektrum:
$\nu_{max}^{CHCl_3}=3450$, 3350, 3250, 2960, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1075, 1020, 985, 955, 905, 850 cm^{-1}

8) Elementaranalyse:
C: 52,03%, H: 5,71%, N: 4,15%, S: 9,86%

9) Dünnschichtchromatographie:

stationäre Phase	Entwicklungs-lösungsmittel	Rf-Wert
Kieselgelfolie	Chloroform: Methanol (10:1) (v/v)	0,59
	Chloroform: Aceton (1:1) (v/v)	0,33

10) Molekulargewicht:
FD-Masse: m/z 1333 (M^++Na)
FABQ-MS: m/z 1311 (M^++1)
Gelp rm ierungschromat graphie: 1100—1200

11) ^{13}C-Nuklearmagnetisches Res nanzspektrum ($CDCl_3$):
δ (ppm): 13,9, 14,6, 16,7, 17,6, 19,9, 22,8, 29,1, 34,0, 35,2, 39,6, 42,3, 52,7, 55,8, 56,1, 56,2, 56,2, 60,3,

61,7, 64,7, 66,7, 68,4, 69,0, 69,3, 69,8, 70,5, 72,0, 75,9, 76,1, 77,2, 77,3, 83,2, 86,3, 88,5, 90,7, 97,3, 98,6, 98,9, 99,6, 99,7, 103,9, 107,8, 112,7, 123,2, 125,0, 130,0, 131,1, 136,8, 144,2, 146,6, 154,0, 154,6, 160,9, 166,6, 192,4, wie in Fig. 1 der begleitenden Abbildung gez igt.

12) ^{1}H-Nuklearmagnetisches Resonanzspektrum ($CDCl_3$):
δ (ppm): 8,57 (1H, s), 7,48 (1H, s), 6,6 (1H, d,d), 6,2 (1H, d, J=1,6Hz), 6,18 (1H, br. s), 5,93 (1H, d, J=9,6Hz), 5,83 (1H, d, d J=9,6, 1,6Hz), 5,7 (1H, d, J=2Hz), 5,5 (1H, m), 5,48 (1H, d, J=2,3Hz), 5,43 (1H, br. s), 4,97 (1H, d), 4,7 (2H, m), 4,56 (1H, d, J=2,3Hz), 4,23 (1H, s), 4,2 bis 3,6 (10 bis 14H), 3,97 (3H, s), 3,88 (3H, s), 3,79 (3H, s), 3,5 (2H, m), 3,43 (3H, s), 2,77 (1H, s), 2,7 (2H, m), 2,52 (3H, s), 2,5 (1H, m), 2,4 bis 2,25 (3H, m), 2,17 (1H, s), 2,12 (3H, s), 2,07 (1H, m), 1,77 (2H, s), 1,5 (2H, m), 1,41 (3H, d, J=6Hz), 1,35 (3H, d, J=6Hz), 1,32 (3H, d, J=6Hz), 1,2 (4H, m), wie in Fig. 2 der begleitenden Abbildung gezeigt; und

ii) Substanz WS 6049-B hat die nachstehenden Eigenschaften:

1) Form und Farbe:
farbloses Pulver

2) Farbreaktion:
positiv: Dragendorff-Reaktion, Ehrlich's Reaktion und Cersulfatreaktion
negativ: Ninhydrin-Reaktion

3) Löslichkeit:
löslich: Methanol, Aceton, Chloroform
wenig löslich: Diethylether
unlöslich: Hexan

4) Schmelzpunkt:
145°C (Zers.)

5) Spezifische Rotation:
$[\alpha]_D^{25}=-201°$ (c=1,0, $CHCl_3$)

6) UV-Absorptionsspektrum:
$\lambda_{max}^{CH_3OH}$=253 nm ($E_{1\,cm}^{1\%}$=620)
=280 nm ($E_{1\,cm}^{1\%}$=450)
=320 nm ($E_{1\,cm}^{1\%}$=250)
$\lambda_{max}^{CH_3OH+HCl}$=253, 280, 320 nm
$\lambda_{max}^{CH_3OH+NaOH}$=250, 282, 310 (sh.) nm

7) IR-Absorptionsspektrum:
$\nu_{max}^{CHCl_3}$=3450, 3350, 3250, 2990, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1070, 1020, 985, 955, 905, 880, 850 cm^{-1}

8) Elementaranalyse:
C: 51,58%, H: 5,75%, N: 4,27%, S: 9,80%

9) Dünnschichtchromatographie:

stationäre Phase	Entwicklungs-lösungsmittel	Rf-Wert
Kieselgelfolie	Chloroform: Methanol (10:1) (v/v)	0,53
	Chloroform: Aceton (1:1) (v/v)	0,18

10) Molekulargewicht:
Gelpermeierungschromatographie: 1100 bis 1200

11) ^{13}C-Nuklearmagnetisches Resonanzspektrum ($CDCl_3$):
δ (ppm): 13,8, 16,7, 17,6, 19,8, 22,7, 29,1, 33,9, 34,1, 35,2, 39,5, 52,8, 55,8, 56,1, 56,3, 61,0, 61,5, 64,6, 66,7, 68,4, 69,0, 69,3, 69,8, 70,4, 71,9, 75,9, 76,2, 76,7, 77,2, 77,3, 77,6, 83,2, 86,6, 88,3, 90,7, 97,3, 98,6, 99,0, 99,6, 99,7, 103,9, 107,8, 112,7, 123,2, 124,9, 129,8, 131,0, 136,8, 144,2, 146,3, 154,0, 154,6, 160,9, 166,6, 192,4, wie in Fig. 3 der begleitenden Abbildung gezeigt.

12) H-Nuklearmagnetisches Resonanzspektrum ($CDCl_3$):
δ (ppm): 11,75 (1H, s), 8,57 (1H, s), 7,48 (1H, s), 6,6 (1H, d, d), 6,24 (1H, d, J=1,3Hz), 6,18 (1H, br. s), 5,93 (1H, d, J=9,2Hz), 5,83 (1H, d, d, J=9Hz und 1,3Hz), 5,70 (1H, br. d), 5,5 (1H, m), 5,47 (1H, d, J=2,3Hz), 5,42 (1H, br. s), 4,98 (1H, d, J=9Hz), 4,7 bis 4,6 (2H, m), 4,56 (1H, d, J=2,3Hz), 4,24 (1H, s), 4,15 bis 3,4 (12 bis 18H), 3,97 (3H, s), 3,88 (3H, s), 3,79 (3H, s), 3,42 (3H, s), 2,52 (3H, s), 2,6 bis 2,4 (2H, m), 2,4 bis 2,2 (7 bis 8H), 2,12 (3H, s), 2,2 bis 2,0 (2H, m), 1,5 (2H, m), 1,4 (3H, d, J=6Hz), wi in Fig. 4 der begleitend n Abbildung gez igt.

2. Verfahren zur Herstellung der Substanzen WS 6049 nach Anspruch 1, ausgewählt aus d r Substanz

WS 6049-A und der Substanz WS 6049-B, welches umfaßt die Kultivierung des Stammes *Actinomadura pulveraceus* sp. nov. No. 6049 (ATCC 39100) in einem wässrigen Nährmedium unter aerobischen Bedingungen und Gewinnung der Substanz WS 6049-A und/oder d r Substanz WS 6049-B.

3. Pharmazeutische Zusamm nsetzung, welche ein n aktiven B standteil, ausgewählt aus WS 6049-A und WS 6049-B nach Anspruch 1 und einen nicht-toxischen, pharmazeutisch annehmbar n Träger umfaßt.

4. Biologisch reine Kultur des Mikroorganismus *Actinomadura pulveraceus* sp. nov. No. 6049 (ATCC 39100).

5. Substanz WS 6049-A, wie in Anspruch 1 definiert, zur Behandlung von Krebs.

6. Substanz WS 6049-B, wie in Anspruch 1 definiert, zur Behandlung von Krebs.

7. Verwendung der Substanzen WS 6049 aus Anspruch 1 zur Herstellung eines Mittels zur Behandlung von Krebs.

Revendications

1. Substances WS 6049 choisies parmi la substance WS 6049-A et la substance WS 6049-B, dans lesquelles:

1) le WS 6049-A présente les propriétés suivantes:
   1) forme et couleur:
      Poudre incolore
   2) Réaction colorée:
      Positive: réaction de Dragendorff, réaction d'Ehrlich et réaction au sulfate de cérium
      Négative: réaction à la ninhydrine.
   3) Solubilité:
      Soluble dans le méthanol, l'acétone, le chloroforme
      Peu insoluble dans l'éther diéthylique
      Insoluble dans l'hexane, l'eau.
   4) Point de fusion:
      150°C (déc.)
   5) Pouvoir rotatoire spécifique:
      $[\alpha]_D^{25}=-208°$ ($c=1{,}0$; $CHCl_3$)
   6) Spectre d'absorption ultraviolette:
      $\lambda_{max}^{CH_3OH}=250$ nm ($E_{1\,cm}^{1\%}=560$)
      $=280$ nm ($E_{1\,cm}^{1\%}=370$)
      $=320$ nm ($E_{1\,cm}^{1\%}=220$)
      $\lambda_{max}^{CH_3OH+HCl}=252$, 280, 320 nm
      $\lambda_{max}^{CH_3OH+NaOH}=250$, 280 env. 310 (épaulement) nm
   7) Spectre d'absorption à l'infrarouge:
      $\nu_{max}^{CHCl_3}=3450$, 3350, 3250, 2960, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1075, 1020, 985, 955, 905, 850 cm^{-1}
   8) Composition élémentaire:
      C: 52,03%, H: 5,71%, N: 4,15%, S: 9,86%
   9) Chromatographie sur couche mince:

Phase stationnaire	Solvant de développement	Rf
Feuille de gel de silice	Chloroforme: méthanol (10:1) (v/v)	0,59
	Chloroforme: acétone (1:1) (v/v)	0,33

   10) Masse moléculare:
      Masse FD: m/z 1333 (M^++Na)
      FABQ MS: m/z 1311 (M^++1)
      Chromatographie par perméation de gel: 1100—1200
   11) Spectre de résonance magnétique nucléaire ^{13}C ($CDCl_3$):
      δ (ppm): 13,9, 14,6, 16,7, 17,6, 19,9, 22,8, 29,1, 34,0, 35,2, 39,6, 42,3, 52,7, 55,8, 56,1, 56,2, 56,2, 60,3, 61,7, 64,7, 66,7, 68,4, 69,0, 69,3, 69,8, 70,5, 72,0, 75,9, 76,1, 77,2, 77,3, 83,2, 86,3, 88,5, 90,7, 97,3, 98,6, 98,9, 99,6, 99,7, 103,9, 107,8, 112,7, 123,2, 125,0, 130,0, 131,1, 136,8, 144,2, 146,6, 154,0, 154,6, 160,9, 166,6, 192,4 comme le m ntre la figur 1 du dessin ann xé.
   12) Spectre de résonance magnétique nucléair ^1H ($CDCl_3$):

δ (ppm): 8,57 (1H, s), 7,48 (1H, s), 6,6 (1H, d.d), 6,2 (1H, d, J=1,6Hz), 6,18 (1H, s large), 5.93 (1H, d, J=9,6Hz), 5,83 (1H, d.d J=9,6, 1,6Hz), 5,7 (1H, d, J=2Hz), 5,5 (1H, m), 5,48 (1H, d, J=2,3Hz), 5,43 (1H, s large), 4,97 (1H, d), 4,7 (2H, m), 4,56 (1H, d, J=2,3Hz), 4,23 (1H, s), 4,2 à 3,6 (10 à 14H), 3,97 (3H, s), 3,88 (3H, s), 3,79 (3H, s), 3,5 (2H, m), 3,43 (3H, s), 2,77 (1H, s), 2,7 (2H, m), 2,52 (3H, s), 2,5 (1H, m), 2,4 à 2,25 (3H, m), 2,17 (1H, s), 2,12 (3H, s), 2,07 (1H, m), 1,77 (2H, s), 1,5 (2H, m), 1,41 (3H, d, J=6Hz), 1,35 (3H, d, J=6Hz), 1,32 (3H, d, J=6Hz), 1,2 (4H, m), comme le montre la figure 2 du dessin annexé et

2) la substance WS 6049-B a les propriétés suivantes:

1) Forme et couleur:
Poudre incolore

2) Réaction colorée:
Positive: réaction de Dragendorff, réaction d'Ehrlich et réaction au sulfate de cérium
Négative: réaction à la ninhydrine

3) Solubilité:
Soluble dans le méthanol, l'acétone, le chloroforme
Peu soluble dans l'éther diéthylique
Insoluble dans l'hexane

4) Point de fusion:
145°C (déc.).

5) Pouvoir rotatoire spécifique:
$[\alpha]_D^{25}=-201°$ (c=1,0, $CHCl_3$)

6) Spectre d'absorption UV:
$\lambda_{max}^{CH_3OH}=253$ nm ($E_{1\,cm}^{1\%}=620$)
$=280$ nm ($E_{1\,cm}^{1\%}=450$)
$=320$ nm ($E_{1\,cm}^{1\%}=250$)
$\lambda_{max}^{CH_3OH+HCl}=253$, 280, 320 nm
$\lambda_{max}^{CH_3OH+NaOH}=250$, 282, 310 (épaulement) nm

7) Spectre d'absorption infrarouge:
$\nu_{max}^{CHCl_3}=3450$, 3350, 3250, 2990, 2920, 1725, 1675, 1610, 1595, 1520, 1465, 1450, 1405, 1370, 1350, 1310, 1250, 1180, 1155, 1115, 1070, 1020, 985, 955, 905, 880, 850 cm^{-1}

8) Composition élémentaire:
C: 51,58%, H: 5,75%, N: 4,27%, S: 9,80%

9) Chromatographie sur couche mince:

Phase stationnaire	Solvant de développement	Rf
Feuille de gel silice	Chloroforme: méthanol (10:1) (v/v)	0,53
	Chloroforme: acétone (1:1) (v/v)	0,18

10) Masse moléculaire:
Chromatographie par perméation de gel: 1100 à 1200

11) Spectre de résonance magnétique nucléaire ^{13}C ($CDCl_3$):
δ (ppm): 13,8, 16,7, 17,6, 19,8, 22,7, 29,1, 33,9, 34,1, 35,2, 39,5, 52,8, 55,8, 56,1, 56,3, 61,0, 61,5, 64,6, 66,7, 68,4, 69,0, 69,3, 69,8, 70,4, 71,9, 75,9, 76,2, 76,7, 77,2, 77,3, 77,6, 83,2, 86,6, 88,3, 90,7, 97,3, 98,6, 99,0, 99,6, 99,7, 103,9, 107,8, 112,7, 123,2, 124,9, 129,8, 131,0, 136,8, 144,2, 146,3, 154,0, 154,6, 160,9, 166,6, 192,4, comme le montre la figure 3 du dessin annexé.

12) Spectre de résonance magnétique nucléaire ^1H ($CDCl_3$):
δ (ppm): 11,75 (1H, s), 8,57 (1H, s), 7,48 (1H, s), 6,6 (1H, d.d.), 6,24 (1H, d, J=1,3Hz), 6,18 (1H, s large), 5,93 (1H, d, J=9,2Hz), 5,83 (1H, d.d, J=9Hz et 1,3Hz), 5,70 (1H, d large), 5,5 (1H, m), 5,47 (1H, d, J=2,3Hz), 5,42 (1H, s, large), 4,98 (1H, d, J=9Hz), 4,7 à 4,6 (2H, m), 4,56 (1H, d, J=2,3Hz), 4,24 (1H, s), 4,15 à 3,4 (12 à 18H), 3,97 (3H, s), 3,88 (3H, s), 3,79 (3H, s), 3,42 (3H, s), 2,52 (3H, s), 2,6 à 2,4 (2H, m), 2,4 à 2,2 (7 à 8H), 2,12 (3H, s), 2,2 à 2,0 (2H, m), 1,5 (2H, m), 1,4 (3H, d, J=6Hz), 1,35 (3H, d, J=6Hz), 1,33 (3H, d, J=6Hz), comme le montre la figure 4 du dessin annexé.

2. Pr cédé de préparation de substances WS 6049 selon la r v ndicati n 1, choisies par la substance WS 6049-A et la substance WS 6049-B, qui consist à cultiver la souche *Actinomadura pulveraceus* sp. nov. N° 6049 (ATCC 39100) dans un milieu nutritif aqueux dans des conditions aérobies et à récupér r la substance WS 6049-A et/ou la substance WS 6049-B.

3. Composition pharmaceutique qui comprend un ingrédient actif choisi parmi l WS 6049-A et le WS 6049-B selon la rev ndication 1 et un support non toxiqu , pharmaceutiquement acceptabl .

4. Cultur bi logiquement pur du microorganisme *Actinomadura pulveraceus* sp. nov. N° 6049 (ATCC 39100).

5. Substance WS 6049-A telle que définie dans la revendication 1 pour le traitem nt du cancer.

6. Substance WS 6049-B telle que définie dans la revendication 1 pour le traitement du cancer.

7. Utilisation des substances WS 6049 de la revendication 1 pour préparer un médicament pour le traitement du cancer.

Figure 1. ^{13}C Nuclear magnetic resonance spectrum (in $CDCl_3$) of WS 6049-A substance

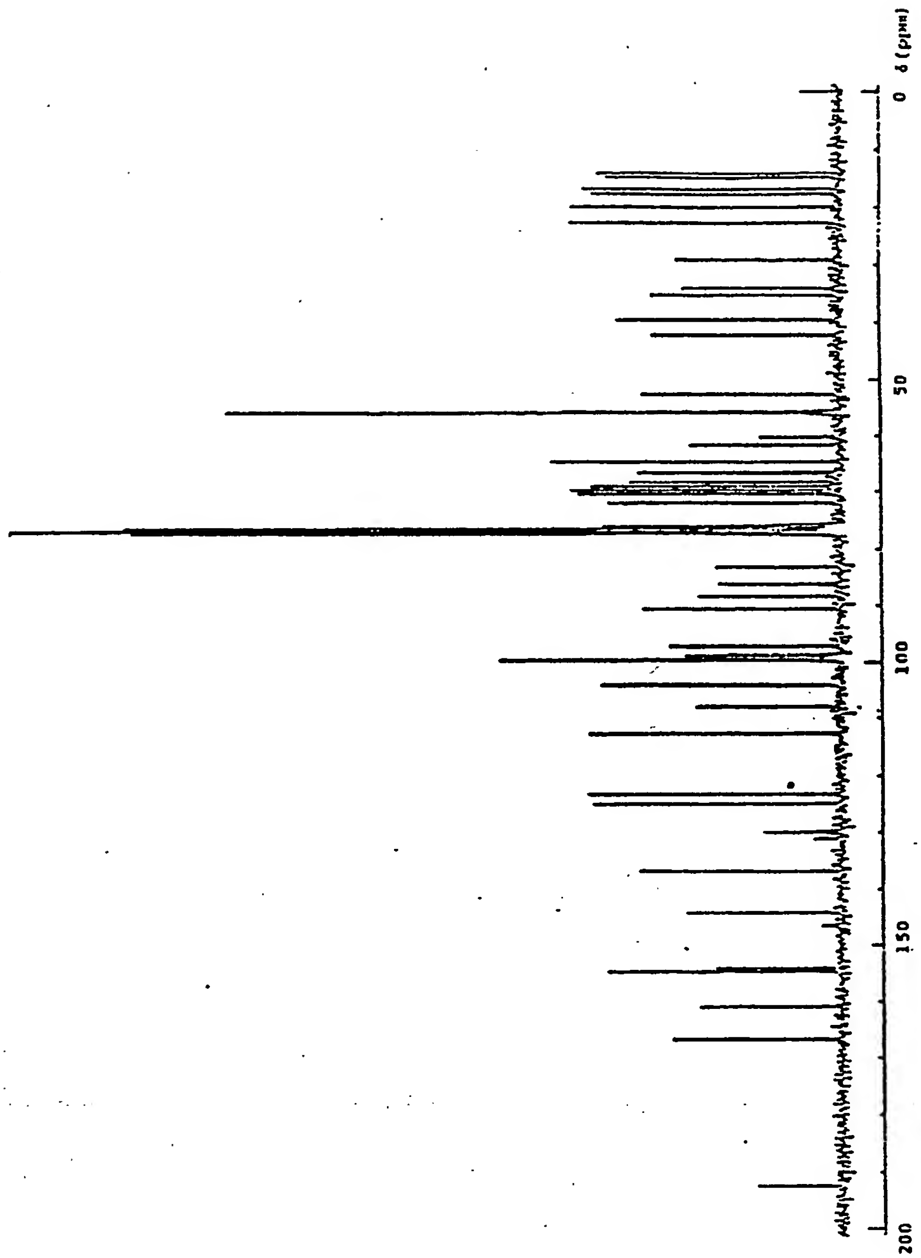


Figure 2. ^{1}H Nuclear magnetic resonance spectrum (in $CDCl_3$) of WS 6049-A substance

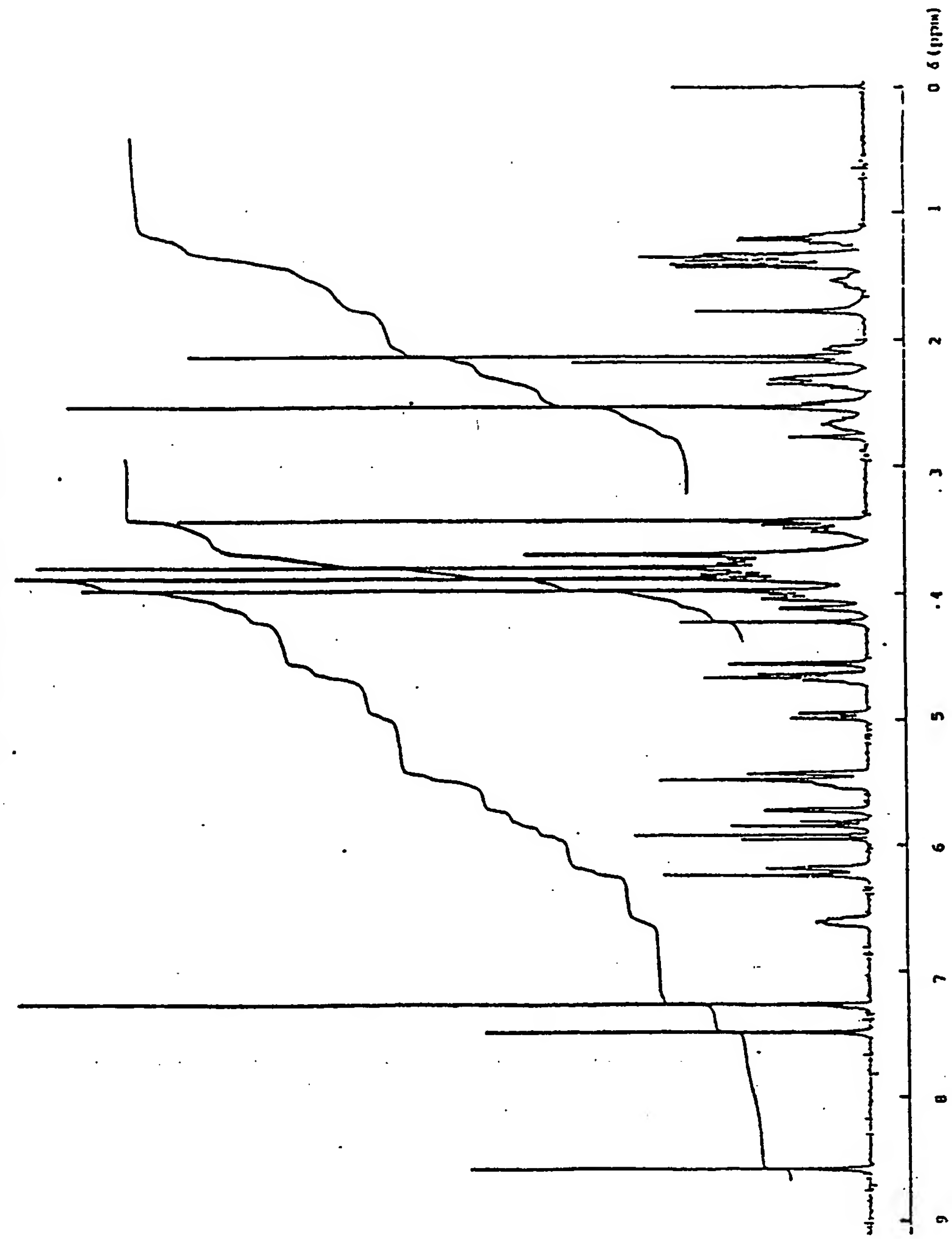
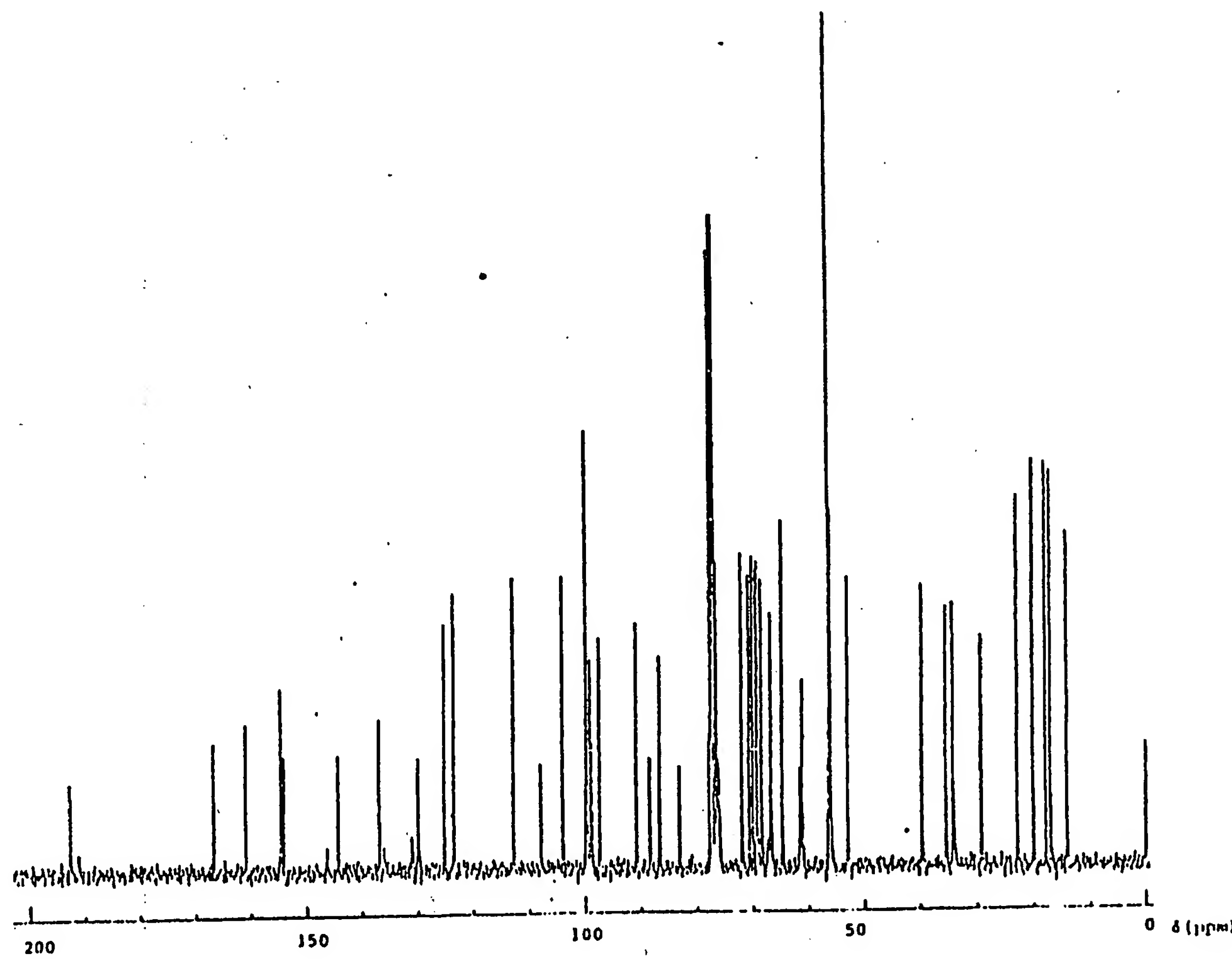


Figure 3. ^{13}C Nuclear magnetic resonance spectrum (in $CDCl_3$) of WS 6049-B substance



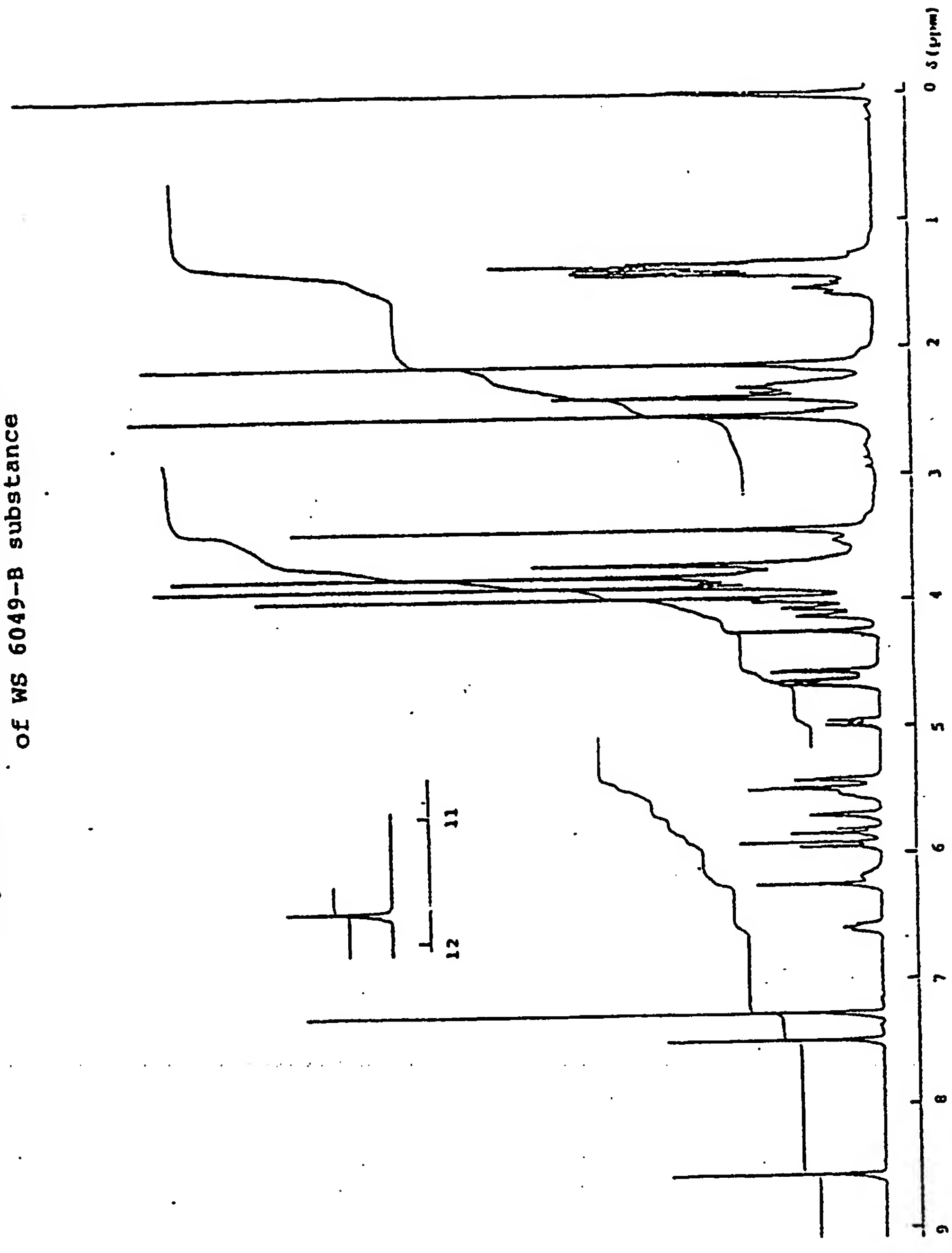


Figure 4. ^1H Nuclear magnetic resonance spectrum (in $CDCl_3$) of WS 6049-B substance